REMARKS

Claims 1-3, 5, 7 and 21-26 are all the claims pending in the application; each of the claims has been rejected.

Applicants respectfully note that while the Office Action Summary sheet does not include claim 21 among the pending claims, as this claim has not been canceled it should be included among the pending claims.

Claim 1 has been amended to provide the full name of the abbreviation "HlyE." Support for the amendment of the claim to recite "hemolysin E" may be found, for example, in the abstract of the Wallace et al. article submitted with the IDS in this application on October 5, 2004 (Cell 100:265-276 (2000)).

The claims have been amended to recite specific bacteria species that can be used in the recited methods. Support for the amendment can be found in paragraphs 0068 and 0069 of the specification.

The Examiner acknowledged the specification supports recitation of a fusion protein comprising an export protein and a protein of interest arranged in a 5' to 3' arrangement (page 4 of the Office Action dated February 10, 2005) as recited in amended claims 1, 22, 24 and 25.

While references to the "*E. coli clyA*" gene were corrected in the specification to be "*E. coli hlyE*" via the Amendment filed October 5, 2004, one such reference was overlooked. Therefore, the instant amendment seeks to correct reference to "*E. coli clyA*" at paragraph 0028, line 9, to properly refer to "*E. coli hlyE*". Support for the amendment may be found at paragraph 0024, lines 6-7, of the specification.

No new matter has been added. Entry of the Amendment is respectfully requested.

I. Priority

At page 2 of the Office Action, the Examiner states that the provisional application (USSN 60/252,516, filed 11/22/2000), of which the instant application claims benefit, fails to adequately support the claims of the pending application.

The Examiner states that the provisional application only indicates that the ClyA protein has potential as a novel secretory protein capable of exporting passenger proteins. The Examiner contends that such disclosure does not provide adequate support for the methods of producing export proteins as recited in the pending claims. More specifically, the Examiner states that the provisional application does not teach any of the sequences recited in the claims (i.e., SEQ ID NOs:2, 23 and 25).

Applicant maintains the position that each pending claim is enabled by the provisional application, and that each claim has adequate written description support in the provisional application.

As noted in the Amendment filed October 5, 2004, the method recited in the independent claims is discussed at pages 8-9 of the provisional application. Sufficient data is provided therein to suggest that the complete method would produce an exported fusion protein. Further, the additional steps needed to practice the method recited in the pending claims are discussed in the provisional application. Evidence of a completed, working example of the claimed method is not required in order for the claims to be enabled.

The test for enablement is whether undue experimentation would be required to practice the invention being claimed. Undue experimentation would not be required to practice the claimed methods as (a) a specific export proteins is identified (*S.* Typhi ClyA), (b) the manner in

which the export protein can be obtained and used is provided, (c) suitable reagents (such as a suitable vector and bacteria) are disclosed, and (d) each of the steps of the method are described in the provisional application.

Furthermore, the export protein sequences were well known in the art at the time the provisional application was filed. Indeed, the sequence of the *E. coli* HlyE protein was incorporated by reference into the provisional application. In particular, Ludwig et al. (*Mol. Micro.* 31(2):557-567 (1999)) is discussed and referenced by incorporation at page 6, lines 9-11, of the provisional application. Ludwig discusses the *E. coli* ClyA protein (referred to in the pending application as HlyE) and references the GenBank entry (U00096) that encodes the *E. coli* genome as the K-12 genome at page 565, column 1, paragraph entitled "construction of plasmids", line 5. Ludwig provides the accession number for the GenBank entry containing the sequence of the K-12 genome at line 21 of the same paragraph.

As can be seen from the enclosed print-out of a portion of the GenBank entry for U00096, the sequence for the hlyE gene is included in the sequence. This sequence is identical to the sequence encoded by the polynucleotide of the *E. coli* hlyE gene shown in SEQ ID NO: 25.

Accordingly, Applicant maintains that each of the pending claims is adequately supported by the disclosure of the provisional application.

II. Claim Rejection Under 35 U.S.C. §112

A. At page 4 of the Office Action, the rejection of claims 1-3, 5 and 7 has been maintained, and applied to claims 21-26, under 35 U.S.C. §112, first paragraph, for reasons of non-enablement.

The Examiner continues to argue that the specification is only enabling for a method of producing a fusion protein, comprising the fusion of the *S. typhi* ClyA protein and a protein of interest (in a 5' to 3' arrangement), in *S. typhi*. The Examiner does not find the use of other *clyA* genes or the use of other bacteria to be enabled. The Examiner explains that there is no showing that *any* hemolysin E is secreted from *any* gram negative bacteria.

Applicant respectfully notes that in contrast to the Examiner's position, Applicant is not claiming the use of *any* HlyE family member. Instead, Applicant is only reciting the use of one of three HlyE family members. Furthermore, as shown in Appendix I of Dr. Galen's first Declaration (submitted with the Amendment in this application on October 5, 2004), the three proteins are very highly homologous. *S.* Typhi and *S. paratyphi* ClyA share 96.4% homology, while *S.* Typhi ClyA and *E. coli* HlyE share 90.5% homology. In view of the limited number of HlyE family members recited in the claims, and their high degree of homology, the skilled artisan would be enabled to practice the full scope of this aspect of the invention as recited.

Applicant also notes that included herewith are amendments to the claims, such that they recite fusion of the export protein to the protein of interest in a 5' to 3' arrangement. The Examiner has acknowledged that fusion proteins so constructed are enabled.

Further included herewith is a publication of the inventor's own work (Trends in Microbiology, 9(8):372-376 (2001)), showing that a fusion protein comprising the *S. Typhi* ClyA export protein linked to the SacB protein, in a 5' to 3' arrangement, is successfully exported from *E. coli* (see page 375, column 1, latter half of first full paragraph, as indicated). Applicant also notes that to further prosecution of this application, the claims are being amended to recite the

use of *S.* Typhi and *E. coli* as the bacteria that may be used in the methods recited in the pending claims.

Thus, while the Examiner states that there is no showing that *any* hemolysin E is secreted from *any* gram negative bacteria, the claims have been amended such that they recite only three highly homologues hemolysin E proteins and the use of only two bacteria (both of which have been shown to export the fusion proteins).

The Examiner further states that published evidence related to non-hemolytic HlyE family member mutations is not relevant because while the published evidence relates to *E. coli* HlyE, claim 24 is directed to mutation of the *S.* Typhi ClyA protein. Moreover, the Examiner states that the evidence shown in Appendix II of Dr. Galen's first Declaration (submitted with the Amendment in this application on October 5, 2004) supports the Examiner's position that success with one type of mutant protein cannot be used to predict success with a different mutant.

Applicant notes that the skilled artisan would expect highly homologous proteins having mutations *in the same residues* to have the same activity. The double mutant had an alteration in positions 187 and 188, while the triple mutant has alternations in positions 185, 187 and 193, the latter having the same residues as recited in claim 24.

In view of the comments above, and the amendments to the claims, Applicant respectfully asserts that the claims are fully enabled, and requests reconsideration and withdrawal of the rejection.

B. At the top of page 9 of the Office Action, claims 1-3, 7 and 21-26 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

The Examiner states that it is unclear whether the phrase "...protein (SEQ ID NO:X)..." limits the indicated protein to the particular sequence.

Included herewith are amendments to the claims to more clearly indicate that the claims are limited to the specific sequences that were recited in the parentheticals of the claims.

The Examiner also states that claim 1 should be amended to provide the entirety of the term "HlyE." Claim 1 has been amended as requested by the Examiner. The skilled artisan would understand "HlyE" to refer to "hemolysin E", as supported by the abstract of the Wallace et al. article submitted with the IDS in this application on October 5, 2004 (Cell 100:265-276 (2000)).

C. At the bottom of page 9 of the Office Action, claims 1-3, 5, 7 and 21-26 are rejected under 35 U.S.C. §112, first paragraph, as lacking adequate written description support in the specification.

The basis for the rejection is that the claims recite SEQ ID NOs:24 and 28, and for the reasons discussed as follows, the Examiner considers such a recitation to be an introduction of new matter. The Examiner also considers introduction of the protein sequences into the Sequence Listing to be new matter.

The Examiner states that amendment of the specification to incorporate subject matter previously only referred to by reference requires the submission of a declaration stating that the material being introduced consists of the same material incorporated by reference at the time of filing.

More specifically, the Examiner requires a Declaration stating that the sequences copied into the Sequence Listing filed October 5, 2004 (referenced by accession number in paragraph

0028 of the specification) are the same as the sequences present in GENBANK at the time the application was filed. The Examiner contends that the GENBANK sequences could have changed since the time the application was filed, and thus requires a Declaration to the effect that they have not changed.

Included in the second Declaration of James E. Galen, filed herewith, is a statement that none of the four polynucleotide sequences incorporated into the Sequence Listing was changed after the date the instant application was filed (November 23, 2001).

Also included with the instant Amendment is a substitute Sequence Listing that does not include the noted polypeptide sequences. In addition, the claims have been amended to recite only the polynucleotide sequences, as suggested by the Examiner. Because the skilled artisan could easily translate a cDNA sequence to arrive at the polypeptide sequence encoded thereby, the specification fully supports the amino acid sequences recited in the claims introduced via the Amendment of October 5, 2004. However, to further prosecution of the application, the claims are being amended as suggested by the Examiner to remove the polypeptide sequences.

In view of these comments, and the amendment to the claims, it is clear that no new matter has been added to the application. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

D. At page 10 of the Office Action, claims 1-3, 5, 7 and 21-26 are also rejected under 35 U.S.C. §112, first paragraph, as lacking adequate written description support in the specification.

The Examiner states that there is no support in the provisional application and instant application for recitation of "gram-negative bacteria." Specifically, the Examiner states

reference to only two gram negative bacteria (*Salmonella* and *E. coli*) is not sufficient support for the sub-genus "gram-negative bacteria."

Included herewith is an amendment of the claims such that they recite the use of *Salmonella enterica* serovariant Typhi (*S.* Typhi) and *E. coli* in place of "gram-negative bacteria." As the Examiner has indicated that the specification fully supports the use of these two bacteria (further supported at paragraphs 0068 and 0069), the amended claims have adequate written description support in the specification and therefore Applicant respectfully requests reconsideration and withdrawal of this rejection.

III. Claim Rejection Under 35 U.S.C. §102

A. At page 7 of the Office Action, the rejection of claims 1, 3 and 7 under 35 U.S.C. §102(b) as being anticipated by Gentschev et al. (Behring Inst Mitt. 98:103-113 (1997)) has been maintained.

The Examiner states that the claims do not "clearly and unambiguously" indicate whether the claims are limited to the specific sequences recited in the claims, or instead recite the specific sequences as "mere examples of the protein." Therefore, the Examiner contends that the claims are not distinguished from the disclosure of the cited art.

Included herewith is an amendment to claim 1 such that it more clearly indicates that only one of two specific polypeptides are used in the claimed methods. Claim 5 merely recites a preferred embodiment of the invention recited in claim 1. Neither of the two specific polypeptides recited in the claim is disclosed in the cited art.

In view of these comments, and the claim amendments, it is clear that Gentschev et al. does not teach each element of the noted claims and thus does not anticipate the noted claims. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

B. At page 11 of the Office Action, claims 1, 3 and 7 are rejected under 35 U.S.C. §102(a) as being anticipated by Wallace et al. (Cell 100:265-276 (2000)).

The Examiner states that Wallace et al. teaches the expression of a GST-HlyE (*E. coli*) fusion protein in an *E. coli* host, and that the fusion protein is inherently secreted, absent convincing evidence to the contrary.

The GST-HlyE fusion protein of Wallace et al. is a fusion of an export protein (HlyE) and a protein of interest (GST) in a 3' to 5' arrangement (see page 265, column 2, lines 3-5 from the bottom). In contrast, the pending claims (as amended herein) recite a fusion of an export protein and a protein of interest in the reverse orientation , i.e., a 5' to 3' arrangement. Therefore, Wallace et al. does not recite each element of the claims (as amended herein) and does not anticipate the noted claims. Applicant respectfully requests reconsideration and withdrawal of this rejection.

IV. Claim Rejection Under 35 U.S.C. §103

At page 7 of the Office Action, the rejection of claims 1-3 and 7 under 35 U.S.C. §103(a) as being unpatentable over Gentschev et al., in view of Curtis et al. (US Patent No. 5,387,744, issued February 7, 1995), has been maintained.

The Examiner notes that because the rejection of the claims over Gentschev et al. has been maintained, the instant rejection has been maintained as well.

As noted above, included herewith is an amendment to claim 1 such that it more clearly indicates that only one of two specific polypeptides are used in the claimed methods. Neither of the two specific polypeptides recited in the claim is disclosed in Gentschev et al. Nor does Curtis et al., alone or in combination with Gentschev et al., teach the method recited in claim 1.

Accordingly, the noted claims are not obvious over Gentschev et al., in view of Curtis et al., and Applicant respectfully requests reconsideration and withdrawal of this rejection.

V. New Matter Rejection

A. At page 8 of the Office Action, the Amendment filed October 5, 2004, is objected to under 35 U.S.C. §132 as introducing new matter into the specification.

The Examiner states that amendment of the specification to incorporate subject matter previously only referred to by reference requires the submission of a declaration stating that the material being introduced consists of the same material incorporated by reference at the time of filing.

More specifically, the Examiner requires a Declaration stating that the sequences copied into the Sequence Listing filed October 5, 2004 (referenced by accession number in paragraph 0028 of the specification) are the same as the sequences present in GENBANK at the time the application was filed. The Examiner contends that the GENBANK sequences could have changed since the time the application was filed, and thus requires a Declaration to the effect that they have not changed.

Included in the second Declaration of James E. Galen, filed herewith, is a statement that none of the four polynucleotide sequences incorporated into the Sequence Listing, and

referenced by sequence identifies in paragraph 0028 of the specification, was changed after the date the instant application was filed (November 23, 2001).

In view of the statements in the second Declaration, reconsideration and withdrawal of this objection is respectfully requested.

B. The Examiner also states at page 8 of the Office Action that the specification only references polynucleotides, and not the polypeptides sequences.

The claims have been amended to recite only the polynucleotide sequences, as suggested by the Examiner. Because the skilled artisan could easily translate a cDNA sequence to arrive at the polypeptide sequence encoded thereby (and the polypeptide sequences are included in the same GENBANK entries as the polynucleotides), the specification fully supports the amino acid sequences recited in the claims introduced via the Amendment of October 5, 2004. However, to further prosecution of the application, the claims are being amended as suggested by the Examiner to remove the polypeptide sequences.

Furthermore, included herewith is a substitute Sequence Listing that does not include the polypeptide sequences. Applicant respectfully requests entry of the substitute Sequence Listing.

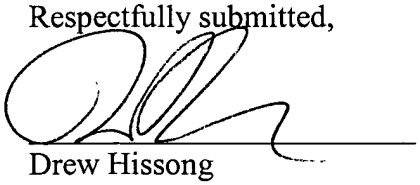
In view of the amendment to the Sequence Listing, Applicant respectfully requests reconsideration and withdrawal of this portion of the rejection.

VI. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Drew Hissong
Registration No. 44,765

Date: July 11, 2005